TRACER STUDIES OF THE BREAKDOWN OF FOREST LITTER

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INTRODUCTION

The great power of radionuclide techniques in ecology can be exploited simultaneously for information on the biogeochemical transfer of an individual element, and as a device for answering broad questions such as those concerning the general rate of flow of energy and the cycling of nutrients in ecosystems. The consumption of food, and hence the transfer of energy and of radionuclides from producing plants to herbivorous and predaceous insects, has been considered by Crossley and Howden (1961), Crossley (1963), and Odum and Golley (1963). Additional steps of transfer to be considered in this paper involve the breakdown of litter by decomposer organisms and the release of radioactive nuclides and of nutrients to the soil, from which they may be recycled through the living parts of the ecosystem.

Techniques of studying this cycle which are to be discussed have been developed as part of a larger study in the Southern Appalachian forests to which many individuals have contributed (Olson et al., 1960, 1961). Shanks and Olson (1961) have examined the differences in breakdown rates of diverse species of unlabeled leaves in contrasting vegetational and climatic conditions of the Oak Ridge Reservation, Oak Ridge, Tennessee, and the nearby Great Smoky Mountains. Crossley and Hoglund (1962) are relating the populations of mites and insects to the weight loss and nuclide release of the litter on which they feed. Witkamp (1960) has recently extended several methods of following the microbial populations, respiration, and growth rates which he studied on a long-range project on litter breakdown at Arnhem in the Netherlands. (Witkamp, 1960).

In England, Murphy (no date) utilized tantalum-182 primarily as a label for identification of and recovery of leaves, the breakdown of which was studied by weighing. This technique has one advantage of permitting free movement of leaves in the litter, and offers interesting possibilities for ecological studies of dispersal by animals or by wind or water. Our own early work has mainly utilized several variations of what we call the "litter bag" technique in order to concentrate attention on the fate of a definite amount of confined litter with a definite initial amount of nuclide. However, we appreciate the limitations imposed by such artificial confinement of litter, and other studies with unbagged litter are in progress.

METHODS

The litter bag technique is an extension of the use of hairnets for confining leaves, which was employed extensively in a program of the Nature Conservancy in England (Bocock and Gilbert, 1957; Bocock et al., 1960). Studies of Shanks and Olson (1961) utilized nylon netting of the same type found in ballroom dresses, and found it fairly satisfactory for experiments lasting one or two years. Mesh size was smaller (2.3 millimeters) than that of hairnets and thereby restrained removal of large fragments of litter by animals, but bags could be sewed to sizes large enough (e.g., 40 by 40 centimeters, 20 by 20 centimeters effective area) to permit substantial mats of forest floor materials to develop in their usual horizontal orientation.

Weighed amounts of air-dried leaves (collected from trees in some experiments, or immediately after falling in others) were enclosed in the bags by rust-proof safety pins, which served incidentally as convenient points for inserting a stainless steel five-inch nail to hold the bag in its mapped position on the ground.

While entry of larger animals may have been restrained, these bags developed a high population of smaller animals within a few weeks. Crossley and Hoglund (1962) preferred working with decimeter square bags so that only a few hundred arthropods had to be handled per sample. They suspected that the smaller size permitted more effective extraction of the fauna by the Berlese method than the more massive samples of litter in which arthropods might find retreats where they would remain instead of being driven out through the Tullgren funnel. Two kinds of fiberglass curtain mesh were found to differ slightly from nylon net in their effect on litter bag microclimate, but so far appear more durable than nylon in field performance.

The use of artificially labeled litter avoids most of the difficulties of low-level counting and mixed radionuclide contamination that might interfere with measurements in controlled experiments if materials contaminated with fallout from nuclear tests or waste disposal residues were used.

Graham's (1954) technique of sealing closed vials in holes in the trees prior to releasing the nuclide was used initially and has an advantage of minimal risk of external contamination, but restrictions of small volume require nuclide preparations of high specific activity or special treatment to neutralize excess acidity. A hand pressure pump of the garden spray type (Auerbach et al., 1959; Figure 37) makes possible a rapid intake (three to ten minutes) of 20 to 40 milliliters of solution through pipes threaded into holes in tree trunks, from which pathways of subsequent translocation could be followed. Mauget feeders were more convenient for inserting at many points around a tree, and have led to some cases of rapid uptake, but rate of emptying varies widely. Even with the bottoms of the feeders partially filled to give prompt drainage of solution, several rinses may be needed to get most of it out of the feeder. One of the best methods for rapid absorption of nuclides is a variation of the trough technique of Frase and Mawson (1953), which consists of the construction of a trough with sealing compound and aluminum foil. A chisel is used to cut into the sapwood of the tree below the level of water in the trough. Radionuclide solution and several rinses of water are poured in as rapidly as the solution is sucked into the vascular system by transpiration.

The use of several millicuries of radioactive tracer for trees two to five inches in diameter usually results in high uptake of cesium-134 (often exceeding 10^6 disintegrations per minute per gram). Absolute uptake of other nuclides (strontium-90, strontium-85, calcium-45, cobalt-60, zinc-65, iron-59, ruthenium-106) is usually lower and more variable. Translocation varies greatly with season, weather, location of material in the tree, and with details of techniques.

When labeled leaves are used, the decimeter litter bags have an additional advantage in that repeated counting of the amount of tracer remaining is possible. The litter bag is collected in a plastic bag, inserted into a 4.5 inch plastic sandwich box (Figure 1), and counted in a standardized position over a scintillation crystal. Procedure 1 (Crossley and Hoglund, 1962) involves animal extraction, weighing in standard laboratory air-dry conditions, counting for radioactivity, and returning to the field one week after collection, without disturbance for seven more weeks; repeated analyses on the same bag are scheduled every eight weeks, so that seven cycles cover slightly over one year. Procedure 2 involves leaving the bags undisturbed until they are scheduled for collection, intact counting, and subsequent partition for purposes of microbial culture on one gram aliquots and chemical analysis on the remainder. Procedure 3 involves counting of bags immediately following their use for two hours during litter respiration experiments, and then returning the bags to the field (usually six hours after collection), so that repeated measurements on the same bag can be made with less drastic disturbance than that required for air-drying and animal extraction.

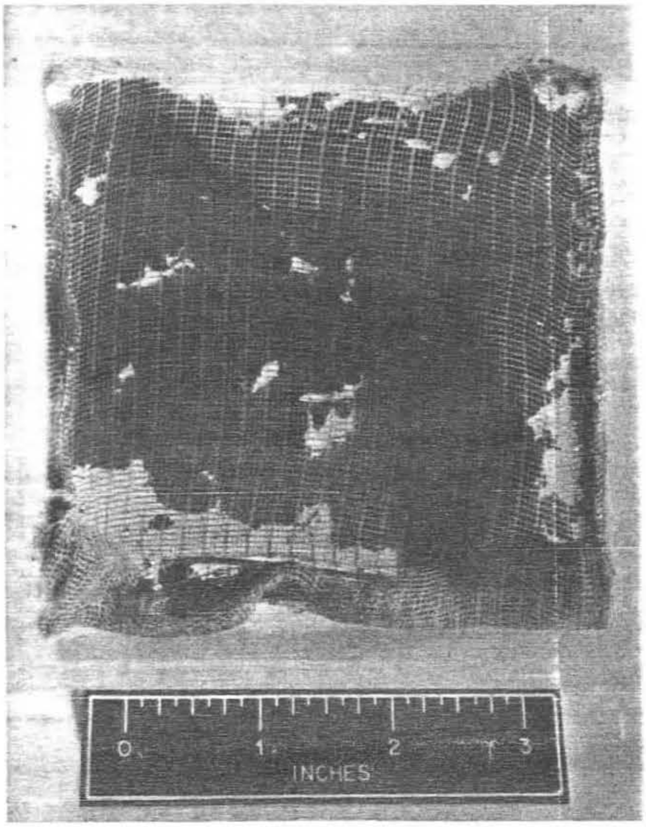

Figure 1. Bag of fiberglass curtain material containing leaves subject to apparently normal breakdown, except for restricted removal of large particles by large animals.

Whereas these techniques merely measure the rate of loss of the nuclides from the litter during its breakdown, the amount of nuclide which enters and is retained in the surface layer of the underlying soil can also be counted using plastic sandwich boxes (Figure 2). Prior collections were made of decimeter square slabs of forest floor material. These cut slabs of forest floor materials were put into plastic dishes which were in turn placed in the field under the bags of labeled litter (Figure 3). Support for the slabs of forest floor was provided by a layer of fiberglass window screen, and contact with the underlying soil through this screen was provided by large holes bored through the bottoms of the plastic box (Figure 4). The whole box was enclosed in a plastic bag for counting. It was inverted so that the ground surface initially receiving the materials being leached from the litter bag rested on a plastic platform that was flush with the aluminum-clad counting scintillation crystal (Figure 4).

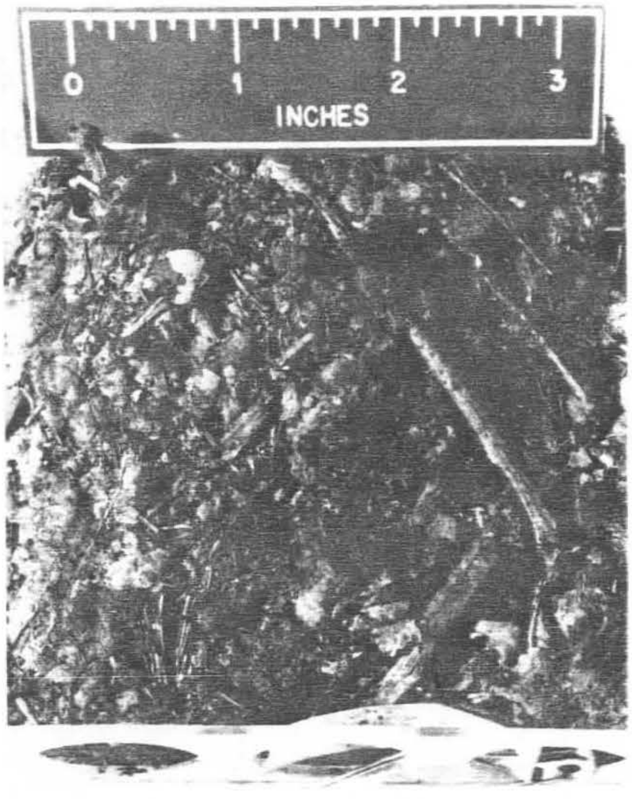

Figure 2. Close-up of old forest floor and humus material undergoing decay underneath the litter bags in the litter boxes.

Because of the problems of standardizing geometry, standards of three configurations (filter

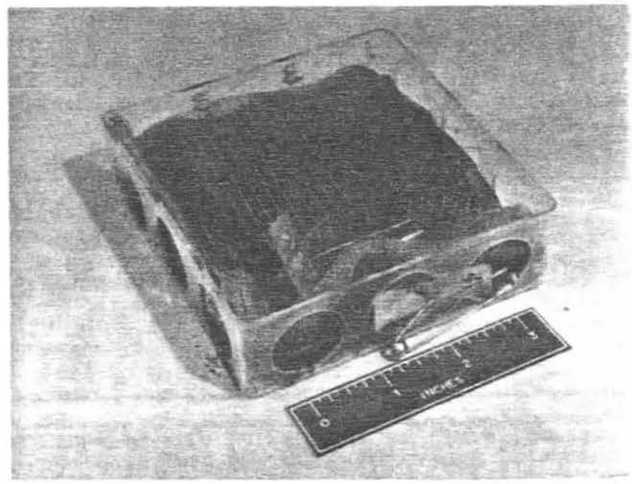

Figure 3. Combination of litter bag nestled in position over litter box.

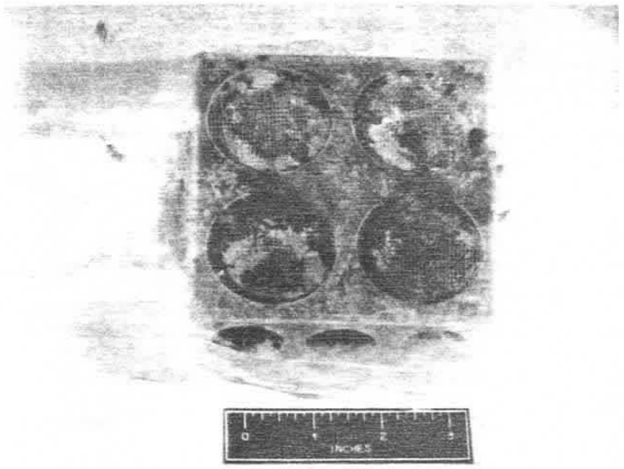

Figure 4. Bottom view of litter box, showing fiberglass window screen material that supports the slab of forest floor material, and holes providing access for movement of small forest animals. Plastic bag for maintaining moisture and nuclides in sample during handling.

paper, 13-millimeter cellulose sponge, 33-millimeter cellulose sponge) were counted along with litter or forest floor slabs (Figure 5). Shifts in horizontal position were minimized by use of a pasteboard positioning device that fits inside the large lead shield surrounding the scintillation detector. In Crossley's later arthropod experiments, effects of varying litter thickness were accounted for by raising the samples slightly as the litter settles, so that the center of gravity of the materials remained fairly constant relative to that of the standard. In other experiments, cellulose sponges were used to press the fresh leaves or the litter into one of the standard geometries. A still better procedure (where low count rate is not a limiting factor) is to position the counting boxes on a platform over the crystal, so that small changes in thickness will have negligible effects.

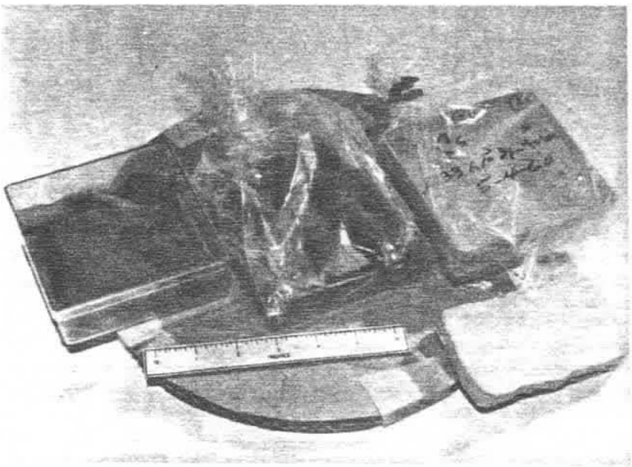

Figure 5. Litter box inverted in cardboard positioner, ready for counting over a scintillation crystal in a large lead shield. Sandwich box containing litter bag on left. Standard with cellulose sponge on upper right. Cellulose sponge for slight compression of loose litter on lower right.

WEIGHT LOSS OF FOREST LITTER

Weight loss can of course be estimated with unlabeled leaves. Shanks and Olson (1961) showed how rates of litter breakdown of deciduous leaves in large litter bags varied systematically as a function of leaf species and the environment.

For example, mulberry (Morus rubra L.) leaves decayed several times as rapidly as beech leaves (*Fagus grandifolia* Ehrh.), while white oak (*Quercus alba* L.), shumard red oak (*Q. shumardii* Buckley), and sugar maple (*Acer saccharum* Marsh.) showed intermediate rates of weight loss. First year losses, averaging over all five species, showed remarkably systematic trends with altitude, with differences in loss rates of approximately 2.4 per cent per thousand feet of elevation in the Southern Appalachian mountains, or about one per cent per year at each of three elevations; differences in rate of weight loss of the same kinds of leaves between typical deciduous and coniferous forests were six per cent. If release of radionuclides and of nutrients is proportional to breakdown of litter, then one might expect from these results to find systematic trends in nuclide release as a function of leaf species, climate, and local differences in forest habitat.

In order to detect seasonal changes in rate of weight loss, weekly weights were taken of labeled pine (*Pinus virginiana* Mill.), oak (*Q. falcata* Michx.), and dogwood (*Cornus florida* L.) leaves in pine and oak forests on the Oak Ridge Reservation. Litter bags were placed in the field

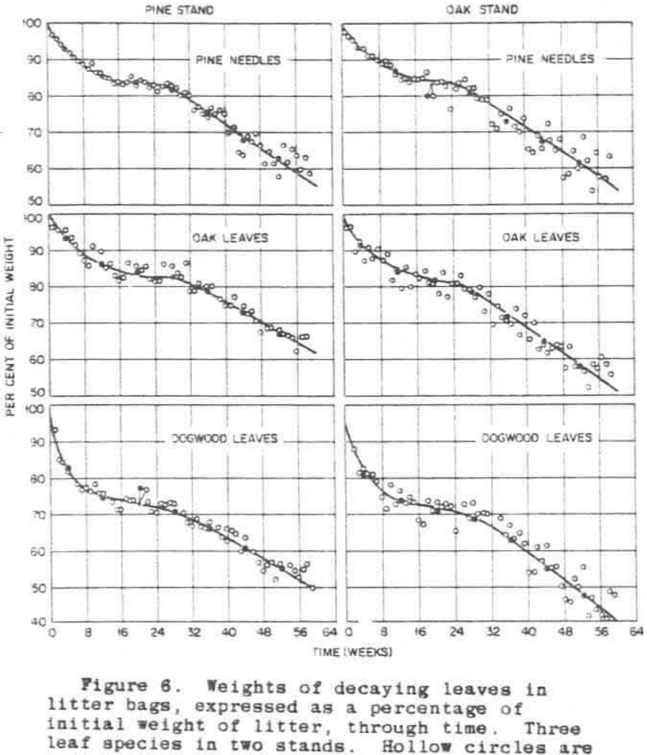

Figure 6. Weights of decaying leaves in litter bags, expressed as a percentage of initial weight of litter, through time. Three leaf species in two stands. Hollow circles are individual measurements; solid circles are averages for eight-week cycles. Lines fitted by eye.

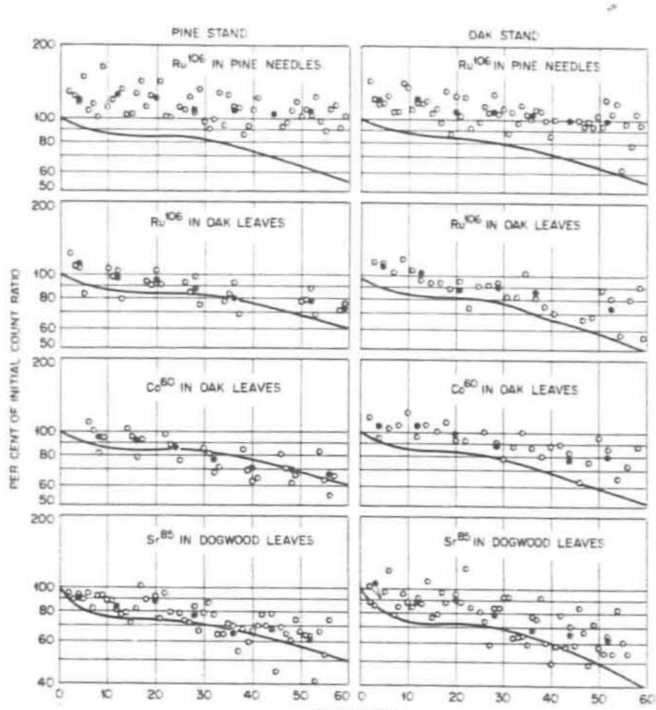

Figure 7. Radionuclide retention by decaying leaves in litter bags, expressed as logarithm to the base 10 of (bag count ratio divided by standard count ratio), as a function of time. Hollow circles are individual measurements; solid circles are averages for eight-week cycles. Solid line represents weight loss replotted on semi-log scale from data of Figure 6. Lines fitted by eye.

in November, 1959, just after the main deciduous litter fall. Procedure 1 was used; i.e., the bags were returned to the field following extraction of arthropods, drying, and weighing, so that repeated measurements were taken on the same bags at eight-week intervals. Figure 6 illustrates the changes in rate of weight loss. Immediately after exposure loss of weight was rapid, particularly so for dogwood. During the winter months the rate of weight loss was slower but was never negligible, even during a cold winter with many periods of snow. The rate of weight loss accelerated during spring and then proceeded at a remarkably steady rate for the rest of the first year. At the end of the first year fractions remaining were: pine, 61 to 63 per cent of the original weight; oak, 58 to 68 per cent; dogwood, 48 to 56 per cent.

Least squares regression coefficients were calculated for the linear regression of weight "fraction of weight remaining" on time, for each of the 48 bags (three species, sampled during eight periods in each of two stands). A log transformation should give a straight line under the hypothetical assumption (Jenny et al., 1949) that litter decay proceeds at a rate proportional to amount of litter remaining. This mathematical model implies that the fraction of litter remaining should follow a negative exponential function e^{-kt}, where k is the rate of breakdown, t is time, and e is the base of the natural logarithms. Such a model does not fit the fine details of curvature shown by the solid line in Figure 7, which was based on the semi-logarithm plot of the weight data from Figure 6. However, the regression procedure provided a surprisingly satisfactory method for fitting a linear first approximation through the curved lines of Figure 7.

Table 1 represents the average of eight regression coefficients for weight loss by litter in the two stands. Time is expressed in years, so that the regression coefficients are estimates of the rate parameter k (0.693 divided by the "half-life of litter breakdown"; see Olson, 1961; Crossley, 1963). The top part of Table 1 is based on the entire first year. For each species breakdown was more rapid in the oak stand; oak and dogwood showed greater discrepancies than did pine. The bottom of Table 1 shows regression coefficients for data with the first two cycles (16 weeks) omitted, so that the sudden early changes in rate of weight loss are ignored. Differences between the two stands are even more accentuated with this treatment. An analysis of variance detected the stand differences as statistically significant but not the difference between species of leaf (Table 1). Real differences exist between rates of breakdown for the different leaf species, but they seem to be more pronounced in the rapid initial loss of weight than in the rate of subsequent breakdown.

Table 1. Average of eight regression coefficients as an estimate of the rate parameter k. Regression of the natural logarithm of weight of remaining litter on time (years). Litter of three leaf species in two stands. Values are natural logarithms of grams per year.

	Pine stand estimate of k	Oak stand estimate of k
	entire year	
Pine needles	0.450 ± 0.0335[1]	0.461 ± 0.0826
Oak leaves	0.330 ± 0.0060	0.484 ± 0.0168
Dogwood leaves	0.421 ± 0.0251	0.560 ± 0.0299
	without first two cycles (16 weeks)	
Pine needles	0.557 ± 0.0563	0.573 ± 0.1018
Oak leaves	0.397 ± 0.0132	0.566 ± 0.0347
Dogwood leaves	0.493 ± 0.0371	0.709 ± 0.0515

Analysis of variance

Source of variation	Degrees of freedom	Mean Square	F
Leaf species	2	0.0000010430	2.13
Stands	1	0.0000044847	9.16*
Species x stands interaction	2	0.0000009272	1.89
Error	42	0.0000004895	
Total	47		

[1] Average ± one standard error of the mean.
*Significant at the 0.01 level of significance, relative to error mean square.

RELEASE OF RADIONUCLIDES

Figure 7 compares the rates of release of ruthenium-106, cobalt-60, and strontium-85 to rates of litter breakdown. Rates of loss of radionuclides were approximately similar to rates of weight loss on the average. However, some distortion of the data resulted from the settling of the initially loose litter, and consequently improved counting geometry, so that counts soon after the early readings were frequently higher than initial counts. It was for this reason that later techniques have reduced this counting difficulty by positioning the litter vertically to a level more nearly equivalent to that of the initial readings. This initial distortion would have obscured any rapid initial loss of radionuclides analogous with the rapid change in weight immediately after exposure in the field.

Table 2. Average regression coefficients for the natural logarithm of bag count rate divided by standard count rate, as a function of time (years). Four radionuclide-litter combinations in two stands. Values are natural logarithms of bags count rate divided by standard count rate.[1]

	Pine stand estimate of k	Oak stand estimate of k
Ruthenium-106 in pine needles	0.81 ± 0.183[2]	0.71 ± 0.192
Ruthenium-106 in oak leaves	1.15 ± 0.531	0.80 ± 0.440
Cobalt-60 in oak leaves	0.56 ± 0.249	0.83 ± 0.326
Strontium-85 in dogwood leaves	0.50 ± 0.117	0.64 ± 0.106

Analysis of variance

Source of variation	Degrees of freedom	Mean Square	F
Leaf species	3	0.0001121521	0.93
Stands	1	0.0000000064	0.00
Species x stands interaction	3	0.0000632518	0.52
Error	40	0.0001208644	
Total	47		

[1] Ruthenium-106 in pine needles and strontium-85 in dogwood leaves based on eight values; the others based on four values.

[2] Average ± one standard error of the mean.

For further indication of subsequent rate of loss of radionuclides from the litter, Table 2 shows regression coefficients (estimates of k) estimated by the same method as for loss of weight. Because of the high variability of individual bags, an analysis of variance failed to detect statistically significant differences between stands or among species of radionuclide for these regression coefficients, but certain trends may be noted from the data. Loss of strontium-90 from dogwood leaves proceeded at a rate approximately similar to the over-all rate of weight (Table 1), whereas loss of ruthenium-106 and cobalt-60 from pine and oak leaves seemed slightly more rapid than loss of weight. The rate of loss of ruthenium-106 seemed more rapid in the pine stand while loss for cobalt-60 and strontium-90 seemed greater in the oak stand. However, improved techniques for counting radioactivity of the litter bags, to reduce the standard errors of such estimates, should permit a firmer detection and estimation of differences in rates of movement of radionuclides.

An example of Procedure 3 for repeated biweekly counting of the same bag of white oak leaves is given in Figure 8. In this case, the nuclide is cesium-134, which evidently is leached much more rapidly than ruthenium, cobalt, or strontium. During the first 32 weeks after the experiment was set out in November, 1960, release of cesium-134 from white oak leaves in a white oak forest averaged a weekly loss of 3.8 per cent of the amount remaining in the preceding week. This is 2.5 times as fast as the average rate of weight loss, which was estimated from biweekly collecting other nearby litter bags by Procedure 2. Final statistics must await analysis of more extensive data still being collected, but these early results suggest a "biological half-life" of only about 17 weeks for cesium-134 in the litter bags, while the "half-life of litter breakdown" was slightly less than one year.[1] In terms of rate parameters discussed in a paper on ecological models (Olson, 1963), these figures are equivalent to values for k of about 2 for cesium-134 release and about 0.8 for litter breakdown, in the example selected for illustration.

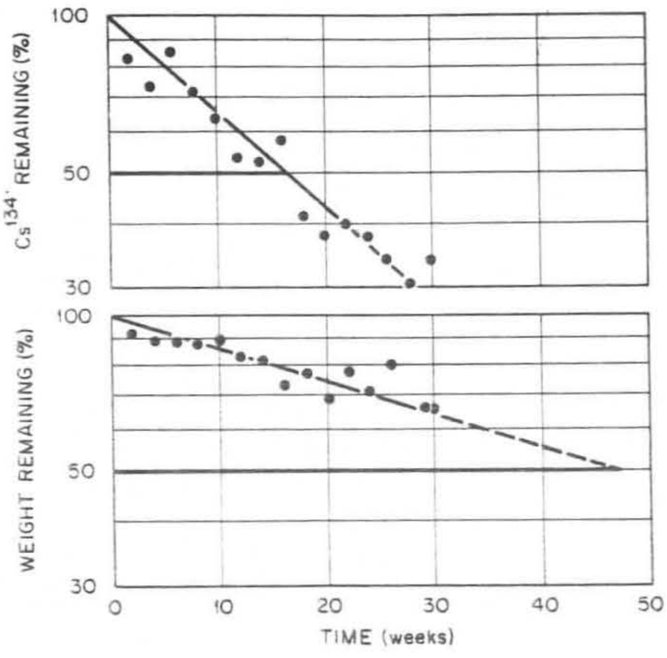

Figure 8. Difference in rate of release of cesium-134 above and weight loss of oak leaves below. Fraction air-dry weight remaining assumed = e^{-kt}. Estimates of k_{cs} = 0.038 and k_{wt} = 0.0154 respectively, pending complete analysis.

Problems of counting geometry and standardization will make it difficult to make an exact comparison between the amounts of cesium-134 leached out of the litter and that retained in the boxes. However, preliminary estimates suggest that about 20 to 30 per cent of the amounts originally estimated for the litter were retained in the underlying slab of forest floor material. Clearly, much more than this amount has leached from the litter (Figure 8), so that there must already have been substantial movement down into the intact portions of the mineral soil profile underlying the forest floor materials.

These examples, then, suggest that a combination of litter-bag and litter-box technique can complement observations on unconfined litter and undisturbed soils. These techniques provide estimates of how rapidly the processes of litter breakdown take place and show that release and subsequent movements of nuclides may be similar to this rate of breakdown in some cases and considerably more rapid in other cases.

SUMMARY

The transfer of chemical elements from fresh dead organic litter to the surface layers of the forest floor to deeper layers of soil and back into living organisms commonly imposes the limiting rate factors on the biogeochemical cycle that occurs within an ecosystem. Radioactive tracers therefore provide powerful methods for investigating the general rate of mineral nutrient turnover as well as the detailed pathways of movement of individual elements and radioactive contaminants.

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[1] Subsequent data at the end of 56 weeks give a regression estimate of k = 0.817 ± 0.031 standard error of the regression estimate. This is equivalent to a half-time of 44 weeks for weight loss.

Previous studies by repeated weighing and chemical analysis of litter have been supplemented by repeated counting of radioactive tracers in standardized counting positions over a scintillation crystal. Labeled litter in decimeter square bags of nylon net or fiberglass curtain material was counted in plastic sandwich boxes. In some cases decimeter square slabs of humus materials, 1.3 to three centimeters thick, were placed in plastic boxes with opening in sides and bottom beneath the litter bags in approximately natural position on the forest floor.

Whereas cobalt-60, ruthenium-106, and strontium-85 or strontium-90 are released from tree-leaf litter at rates similar to or slightly greater than the rate of weight loss, cesium-134 is released considerably more rapidly. Much of the nuclide released is intercepted by the surface slab of humus, especially in types in which mineral soil layers were included. However, there was significant release to lower parts of the soil profile even for these soils.

ACKNOWLEDGMENTS

This work was performed under contract number W-7405-eng-26 between the U. S. Atomic Energy Commission and the Union Carbide Corporation.

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